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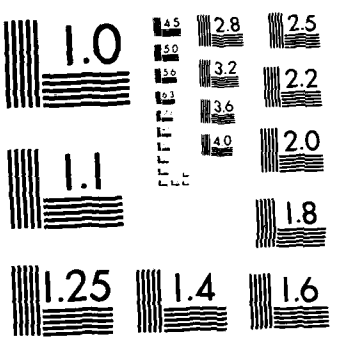

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Isolation and Characterization of Erythrocyte and Parasite Membranes from Rhesus Red Cells Infected with P. knowlesi

Annual Summary Report

Donald F. H. Wallach

June 1, 1979 - May 31, 1980

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

1. Two immunogenic antigens common to host cell membranes of rhesus erythrocytes infected by two strains of P. knowlesi, were shown to be reactive with antibodies from patients acutely exposed to P. falciparum, P. vivax, as well as rhesus or squirrel monkeys made immune to infection with P. cynomolgi and P. falciparum respectively.

2. In rhesus monkeys one of the immune components (component 13) clearly correlates with protective immunity.

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Block 20 (Continued).

3. The immune components are also shared by P. falciparum merozoites and host cell membranes from P. cynomolgi-infected erythrocytes.

4. The data indicate that at least one of the antigens, component 13, is an interspecies antigen.

5. The Plasmodium specific antigens can be labeled metabolically by both amino acids and sugar precursors, showing them to be glycoproteins. Their molecular weights and isoelectric points fall in the ranges 90,000-55,000D and pH4.5-5.2, respectively.

6. The Plasmodium specific antigens described are exposed at the external surfaces of infected erythrocytes.

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1. The membrane pathobiology of malaria has been reviewed in depth (Wallach, 1979).

2. The studies on P. knowlesi-specific antigens in membranes of parasitized rhesus monkey erythrocytes have been extended to the following directions:

2.1 Characterization of interspecies plasmodial antigens

As two immunogenic P. knowlesi-specific antigens of molecular masses of 65,000D and 90,000D were shown to be common to the Philippine and Malaysian strain of P. knowlesi, we have explored the possibility of their possible interspecies character.

Sera of three Gambian individuals and three rhesus monkeys immune against infections with Plasmodium falciparum and Plasmodium knowlesi, respectively, were reacted with Triton X-100-solubilized, ^{125}I-labeled P. knowlesi schizonts and membranes of infected erythrocytes. Indirect immune precipitation with Staphylococcus aureus, Cowan strain I, followed by dodecylsulfate polyacrylamide gel electrophoresis, SDS PAGE, was used to identify interspecies plasmodial antigens which are immunogenic in vivo. Both types of sera specifically precipitated Plasmodium-specific antigens with molecular masses of ∿ 125,000D, ∿ 90,000D and 65,000-50,000D from schizonts and membranes of parasitized erythrocytes. Two lower molecular mass species of ∿ 29,000D and ∿ 16,000D were unspecifically deposited by normal and immune sera. Sequential indirect immune precipitation, incubating the antigens first with human and then with monkey immune serum, as well as inhibition studies with P. falciparum antigen, indicate that the ∿ 90,000D and 65,000-50,000D molecular mass antigens contain a minor proportion of antigens seemingly specific for one Plasmodium species. The presence of protective antibodies against interspecies plasmodial antigens in immune hosts may have implications for future developments of anti-plasmodial vaccines (Schmidt-Ullrich, R., Miller, L. H., Wallach, D.F.H. and Lightholder, J., J. Immunol. in press; 1980)

In the above and in the report by Miller et al (L. H. Miller, J. G. Johnson, R. Schmidt-Ullrich, D. Haynes, D.F.H. Wallach and R. Carter, J. Experimental Medicine, in press; 1980), we have demonstrated that malaria proteins on the surface of merozoites and infected red cells share specificities with at least two primate malarias, Plasmodium knowlesi and P. falciparum. Sera from five Gambian adults who were highly immune to Plasmodium falciparum were used as a reagent to study shared specificities among P. falciparum schizonts and surface proteins on P. knowlesi merozoites. The sera bound to the surface of viable, intact P. knowlesi merozoites, although the sera did not block invasion of rhesus red cells. ^{125}I-lactoperoxidase-labeled surface proteins on merozoites were complexes with the antibody. All major protein bands seen in the electrophoresis of the original Triton extract were bound by the immune sera. Since Gambians have never been exposed to P. knowlesi malaria, the antibodies that reacted with P. knowlesi merozoites must be directed against antigens of another parasite such as P. falciparum. We tested this hypothesis by competition for antibody, in a Gambian serum, between Triton extracted P. falciparum and antigen derived from surface labeled P. knowlesi merozoites. P. falciparum inhibited the reaction, indicating crossreaction between antigens in P. falciparum schizonts and P. knowlesi merozoites.

Further, crossed immune electrophoretic analysis of P. knowlesi schizonts and membranes of infected erythrocytes, both labeled to a specific ^{125}I activity of $\sim 2.10^7$ cpm/mg protein, was employed to test a larger number of sera of monkeys protected against P. knowlesi infection. For 10 monkeys immune against P. knowlesi infections, a correlation could be obtained between protective immunity and high titered antibodies against immune component 13, shown by us before to be strongly immunogenic in rhesus monkeys rendered naturally immune against P. knowlesi infections (Schmidt-Ullrich et al, 1979).

Based on our data on immunological crossreactivity between antibodies against antigens immunogenic in rhesus monkeys infected with P. knowlesi and antisera from individuals chronically infected with P. falciparum or transiently exposed to P.

falciparum and P. vivax we have started to characterize interspecies plasmodial antigens. Purified schizonts and membranes of schizont-infected erythrocytes are being isolated from different species, P. knowlesi and P. cynomolgi in rhesus monkeys, P. falciparum propagated in squirrel (Saimiri Sciureus) monkeys (adapted to monkeys and provided by Dr. C. C. Campbell, Center of Disease Control, Atlanta) and from P. chabaudi (M. Hommel, Dept. Molecular Biology, Harvard Medical School, Boston) grown in Sprague-Dawley rats. Our initial results indicate that the prominent immune component 13 on the red cell membranes of erythrocytes infected with P. knowlesi, detected by antibodies in the sera of individuals immune against P.falciparum and P. vivax is also present on rhesus cells infected with P. cynomolgi and is detected by antibodies in serum of monkeys immune against P. cynomolgi. This correlates with protection of rhesus monkeys against infections with P. cynomolgi after infections and challenges with P. knowlesi, Malaysian strain (to be published).

2.2 Antibodies of rhesus monkeys protected (immunized) against Plasmodium knowlesi antigens in membranes of parasitized erythrocytes.

As noted we have identified two immunogenic P. knowlesi antigens in membranes of infected rhesus erythrocytes, immune component 1 and 13 (Schmidt-Ullrich et al, 1979). These antigens are common to the Malaysian and Philippine strain of P. knowlesi, crossreact with antigens of P. falciparum and P. vivax and are present on purified P. knowlesi schizonts, in membranes of schizont-infected erythrocytes and on merozoites.

To correlate the presence of antibody against immune component 13 (and 1) we have tested sera of 25 rhesus monkeys (provided by Dr. L. H. Miller) obtained either after immunization with P. knowlesi antigen (mixed with adjuvant) and boosting, or by infection/cure and repeated challenge with the same parasite. Triton X-100 solubilized ^{125}I-labeled parasite antigen (specific activity $\sim 5 \cdot 10^7$ cpm/mg protein) was tested by crossed immune electrophoresis against ammonium sulfate-precipitated immunoglobulin. The immunoplates were evaluated by Coomassie blue

(protein staining) and ^{125}I-autoradiography. The immune component was always identified by crossed immune electrophoresis in which the serum in question was mixed with the natural hyperimmune serum that reacts only with antigen 13 (Schmidt-Ullrich et al, 1979).

Table 1. Antibodies against P. knowlesi-induced antigen 13 [a]

A: Vaccination/boosts[b]		B: infection/challenges[c]	
protected	not protected	protected	not protected
2/6	0/10	7/7	0/2

a To be published

b *Plasmodium* antigen injected with complete Freund's adjuvant (CFA) incomplete FA or BCG

c Infections and challenges by intravenous injections of schizont-infected erythrocytes.

Table 1 shows there is correlation between protective immunity and circulating antibodies against immune component 13. All monkeys protected against *P. knowlesi* infections were found to have antibody against component 13; in some animals this was the only antibody detected by direct immune precipitation. In the non-protected monkeys (group B) no antibodies against component 13 could be found. Most of the sera of vaccinated monkeys yielded several immune precipitates against *P. knowlesi* antigens; however, only 2 out of 6 protected monkeys and none of the unprotected monkeys were found to have antibody against component 13.

Vaccination of rhesus monkeys with *P. knowlesi* induces protective immunity which is not necessarily mediated by antibodies against one defined *Plasmodium* antigen. However, there is a positive correlation between protective immunity and antibodies against immune component 13 in monkeys infected and rendered immune after consecutive challenges with the same parasite. In addition we have infected/challenged 4 rhesus monkeys as described and selectively induced antibodies against immune component 13 (and in two monkeys also against antigen 1.)

2.3 Studies using metabolic labeling.

Metabolic labeling of highly synchronized trophozoite- and schizont-infected erythrocytes during short term cultures has allowed us to demonstrate that the intracellular plasmodial parasite can synthesize proteins, glycoproteins and glycolipids and that it can export these molecules into the host cell membrane within 6 hrs. The predominant proteins/glycoproteins labeled lie in the 90,000-45,000D molecular mass region and focus at isoelectric-points between pH 4.5 and pH 5.2. This includes components identified by us before as parasite-specific antigens lying in the host cell membrane. Comparing the relative incorporation of ^{14}C-amino acids ^{14}C-glucosamine into components of identical pI or molecular size in schizonts and membranes of infected erythrocytes, indicates that the assembly of glycoproteins and glycolipids must occur at least in part within or at the surface of the intracellular parasite. The synthesis, assembly and export of parasite-synthesized glycoproteins is currently being studied using a variety of carbohydrate precursors and more detailed time course experiments. Different rates of synthesis at different stages of the parasite are indicated by high ^{14}C-amino acid incorporation into a $\sim$ pI 6.2 protein at the trophozoite but not the schizont stage (R. Schmidt-Ullrich, D.F.H. Wallach and J. Lightholder, Cell Biol. Internat. Reports, in press, 1980.)

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